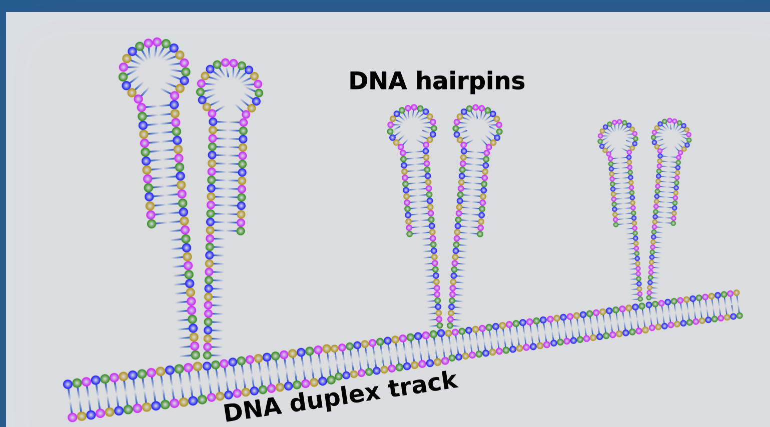
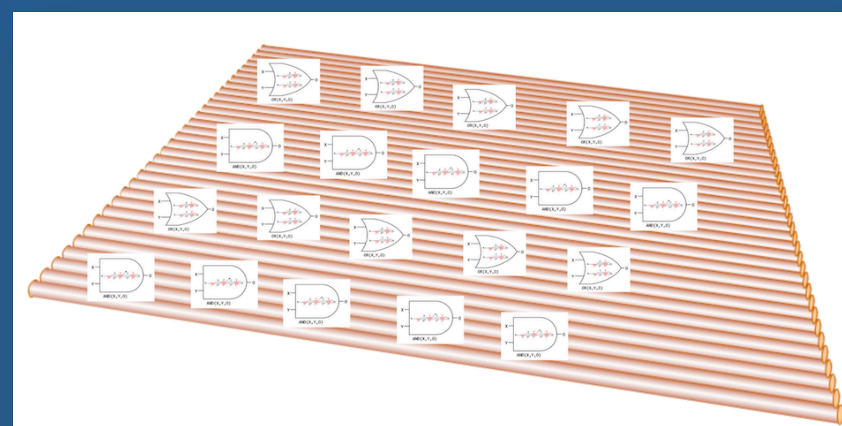
Localized DNA Circuits

Hieu Bui

Outline

- Localized Kinetics & Modelling
- Localized Hybridization Reactions
  - On Nanotracks
  - On DNA Origami





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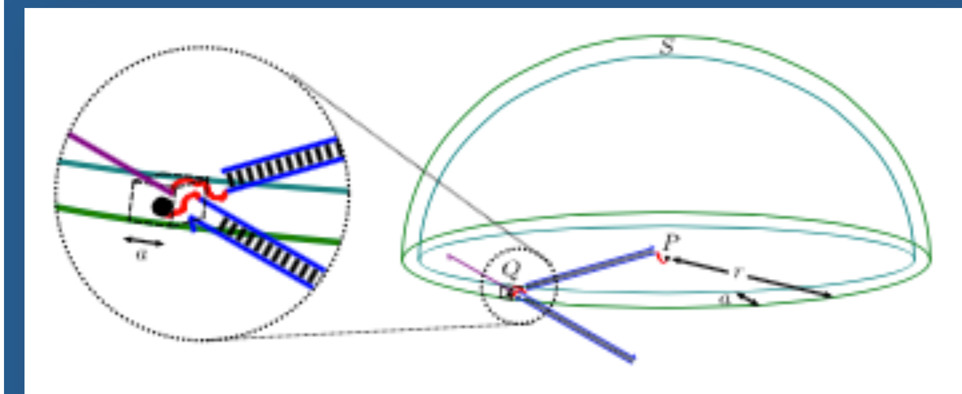
Objective

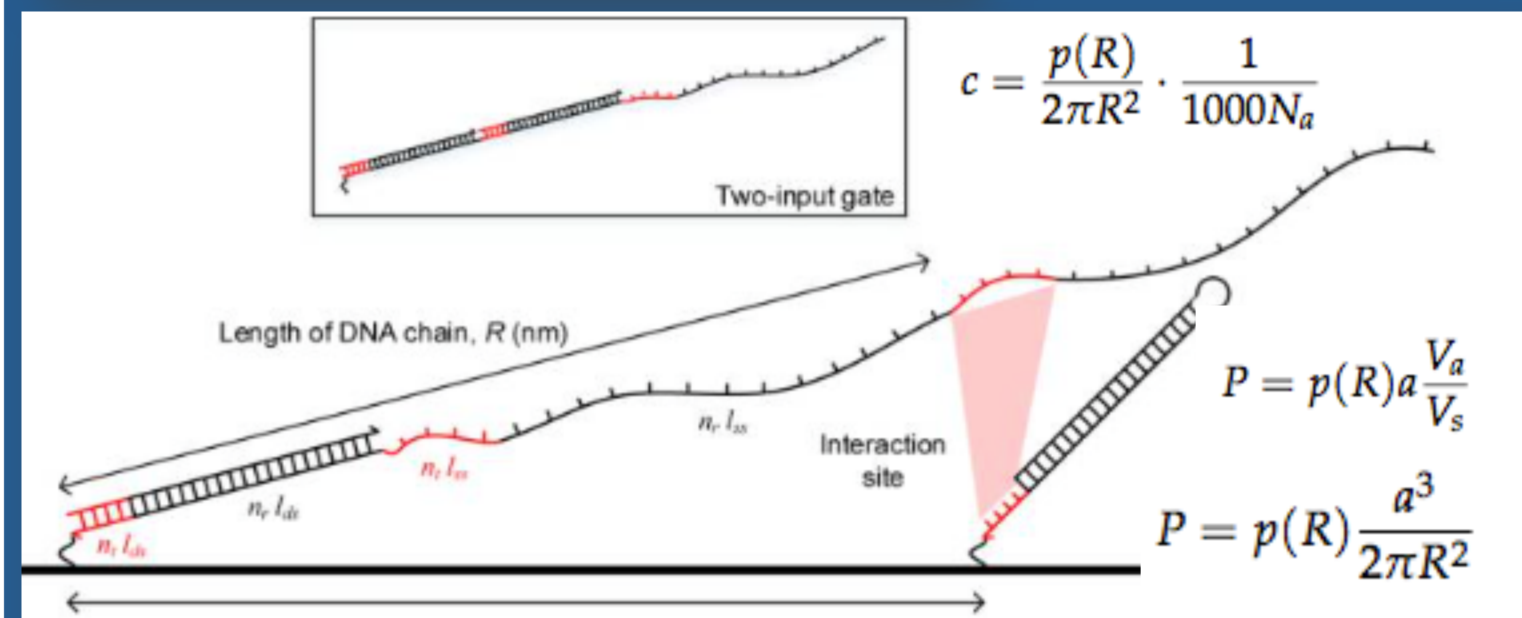
- Challenges from programing DSD devices:
  - Speed
  - Scalability
  - Spurious interactions/crosstalk
- What are the current options?
  - Sequence design (mismatch, clamp, G-C)
  - Lower concentration
- Developing software
  - to ease the sequence design process
  - to track all possible reaction pathways
- Programming DSD devices on 2D surface

A Biophysical Model



Local concentration estimation:

- Distance between molecules
- Flexibility of DNA
- Length of molecules



Software: How to solve it?

- Visual DSD:
  - Special-Purpose: Used to design and analyze DNA strand displacement systems in solution-phase.
  - Not applicable for 2D DNA circuits.
  - Need to employ continuous-time Markov chain (CTMC) to be relevant.
- PRISM:
  - General-Purpose: Used to test logical queries by probabilistic model checking.

Integrate probabilistic model checking within Visual DSD

- Streamline approach to analyze modifications to a given model.
- Optimize the computation of probabilistic queries at multiple time points.

Visual DSD

PRISM

How to calculate transient probabilities?

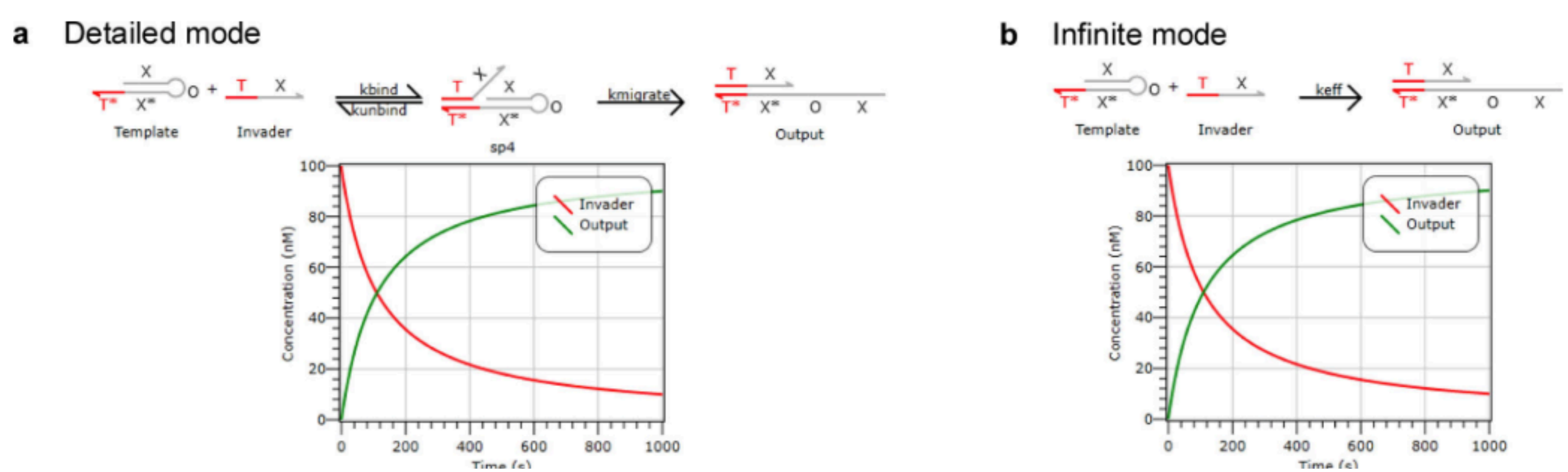
- Visual DSD
  - Numerical integration of the chemical master equation (CME).
  - Propagate the solution at a previous time point to the next one.
  - Low setup cost when requesting multiple time points.
  - May consume large memory to store multiple time points.
- PRISM:
  - Uniformization method: a discrete time conversion of CTMC to calculate the probability of being in a particular state at a particular time.
  - High setup cost when requesting multiple time points.
  - More efficient and stable routine for single calculations.

Chemical Master Equations (CME)

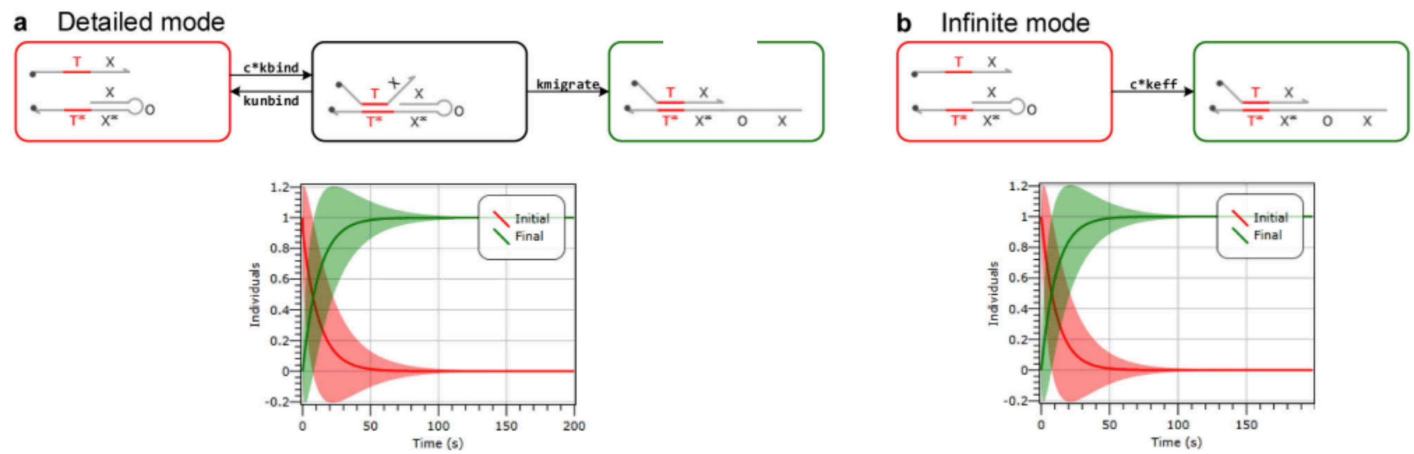
- Distribution of all possible trajectories.
- Analysis of CME:
  - Generate a CTMC (full state space of the system).
  - Each state – a vector of species populations.
  - Each position – a separate species.
- State Space Grows Exponentially with N:
  - Global (non-local) setting:
    - N species and c copies of each species: state space of c^N.
    - Example: N species and 1000 copies of each species: state space of 1000^N.
    - Less efficient due to non-local interactions.
  - Localized setting:
    - N species and a single copy of each species (present or absent), state space of 2^N.
    - More efficient due to localization

Visual DSD

- To simulate the localized strand displacement circuit:
  - Generate a CTMC (For a detailed mode and an infinite mode).
  - Set transition rates

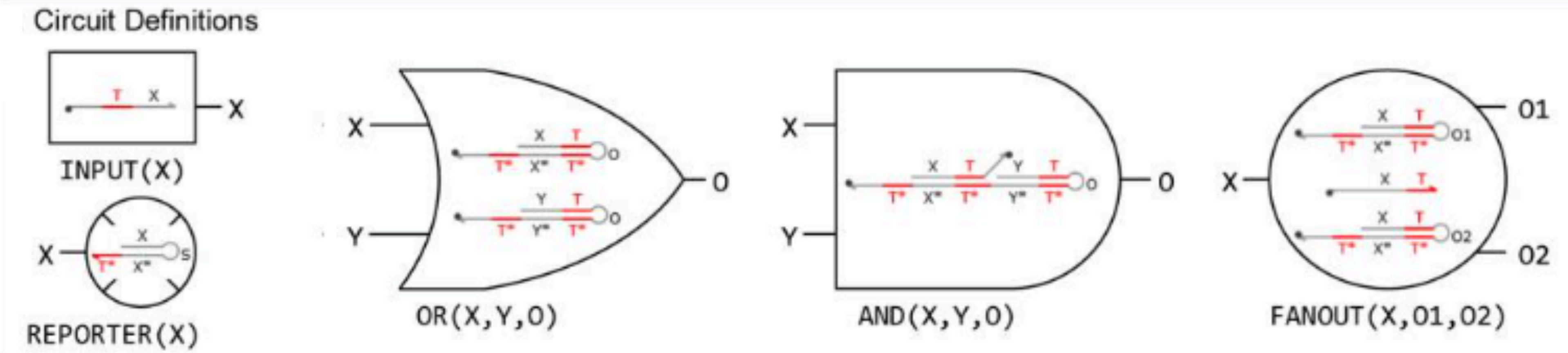


Comparison of compilation modes in Visual DSD. (a) Chemical reaction network (CRN) and ODE simulation for an elementary strand displacement circuit in Detailed mode, in which binding, unbinding, and migration are represented as separate steps. We assume 100 nM of template and invader strands initially, with rates $k_{\text{bind}} = 10^{-3}\ \text{nM}^{-1}\ \text{s}^{-1}$, $k_{\text{unbind}} = 10\ \text{s}^{-1}$, and $k_{\text{migrate}} = 1\ \text{s}^{-1}$. (b) CRN and ODE simulation for the elementary strand displacement circuit in Infinite mode, in which strand displacement is assumed to take place in a single step, with rate $k_{\text{eff}} = k_{\text{bind}} \cdot k_{\text{migrate}} / (k_{\text{migrate}} + k_{\text{unbind}}) = k_{\text{bind}}/11$.

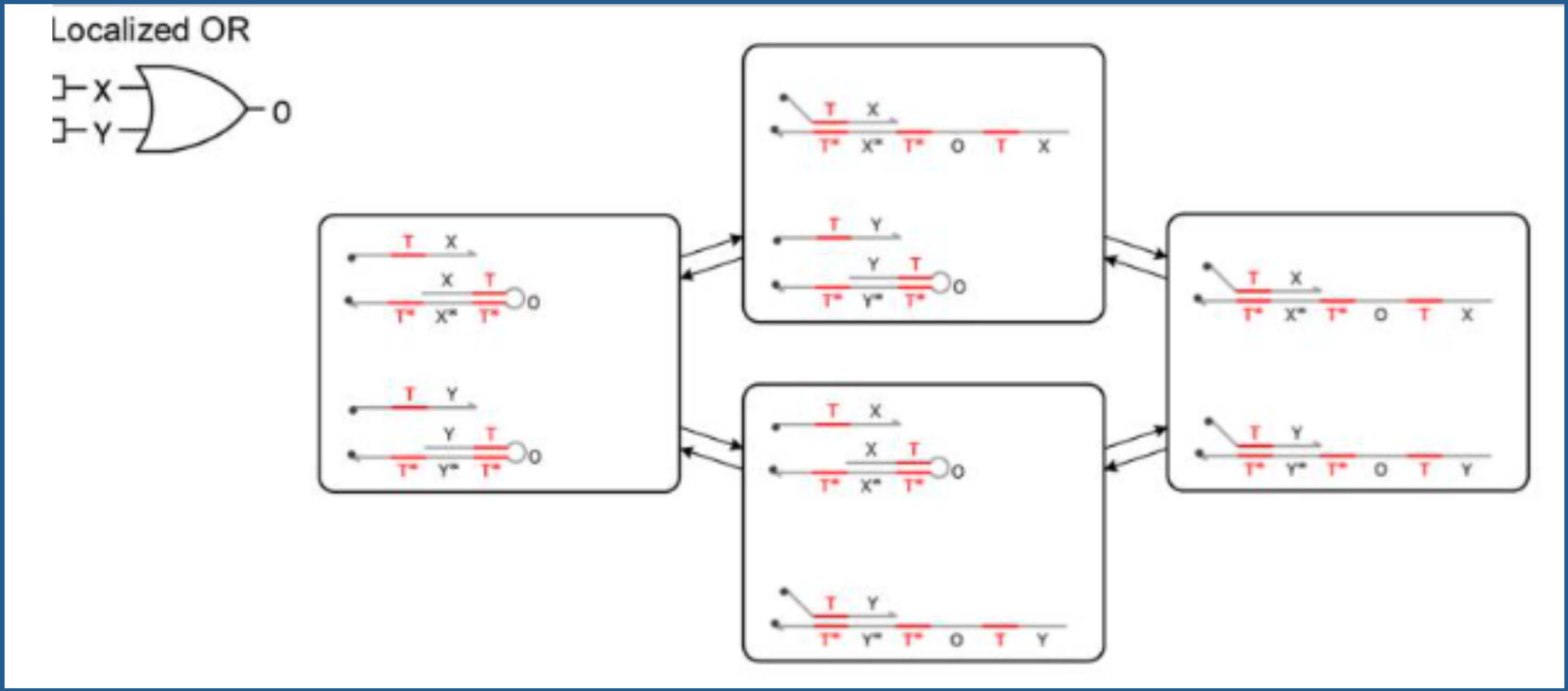


Comparison of compilation modes for localized strand displacement circuits. (a) Continuous-time Markov chain (CTMC) and CME analysis for a localized version of the elementary strand displacement circuit from Figure 1a in Detailed mode. Here, both the invader and template are tethered to the same substrate, in close proximity to one another. The localized binding rate is given by $c \cdot k_{\text{bind}}$, where c is the local concentration, which quantifies the proximity of the two strands. Here, we assume $c = 10^3$ nM. The analysis denotes the probability of the circuit being in a particular state at a given time (solid lines), which corresponds to the expected proportion of circuits in that state, together with the standard deviation for each state (shaded region). (b) CTMC and CME analysis for a localized version of the elementary strand displacement circuit from Figure 1b in Infinite mode.

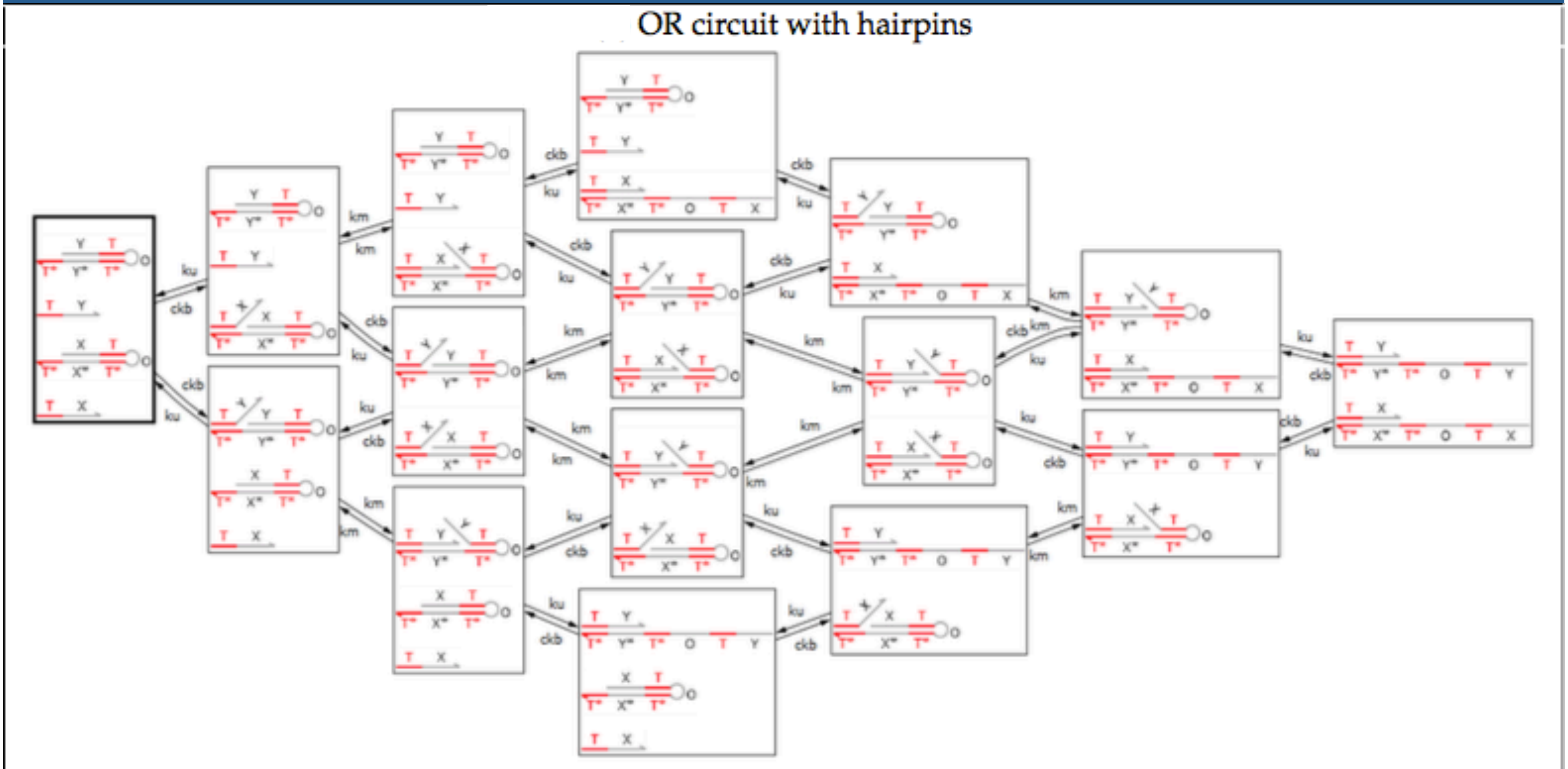
Designed Localized Circuits



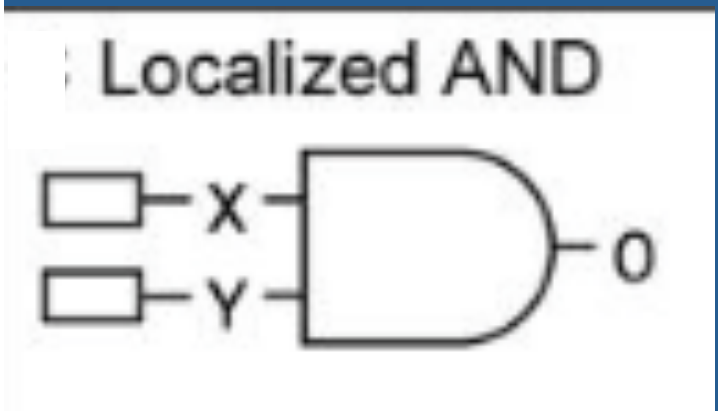
Implementing OR

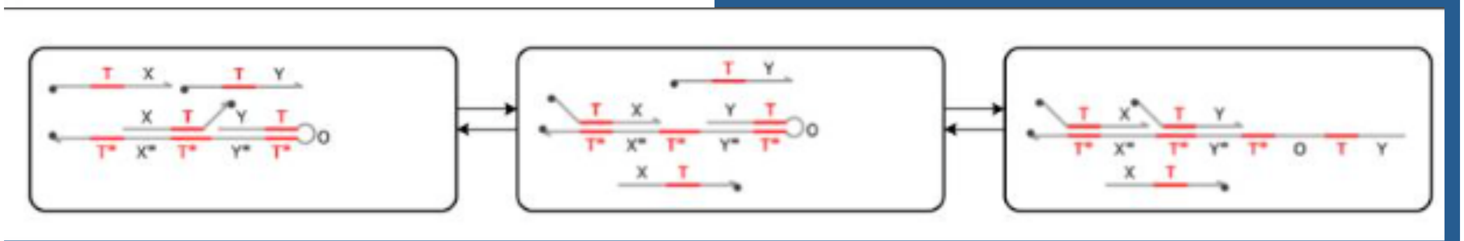


Implementing OR

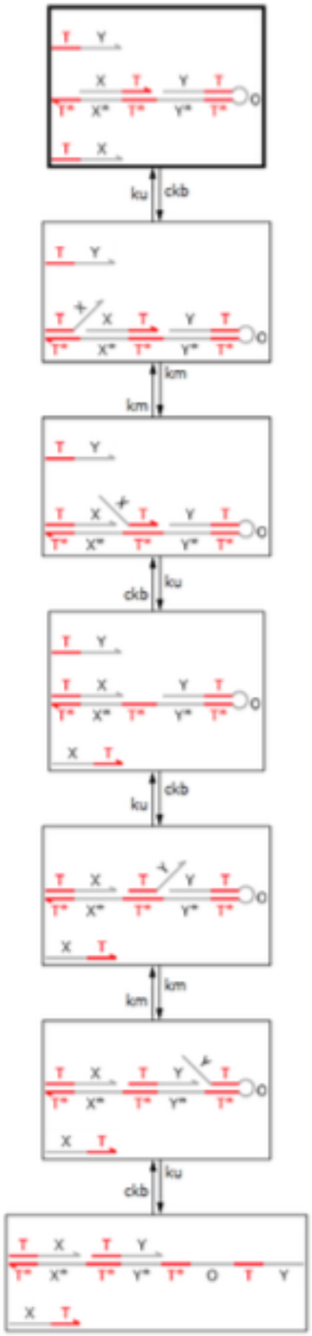


Implementing AND



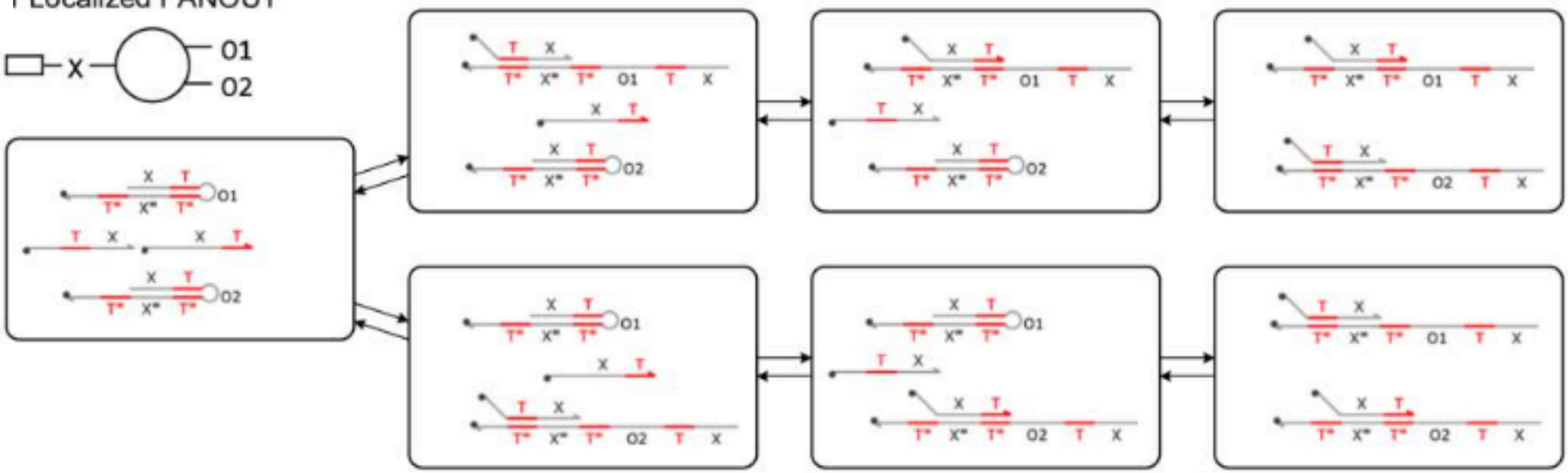


AND circuit with hairpins

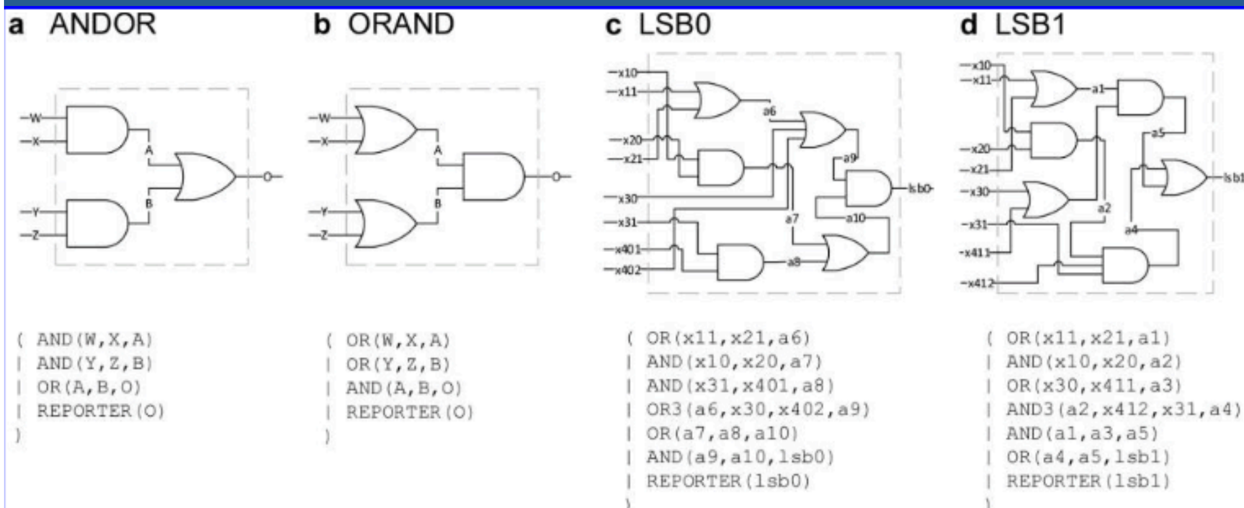


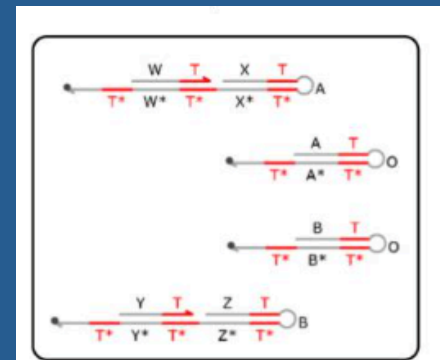
Implementing FANOUT

Localized FANOUT

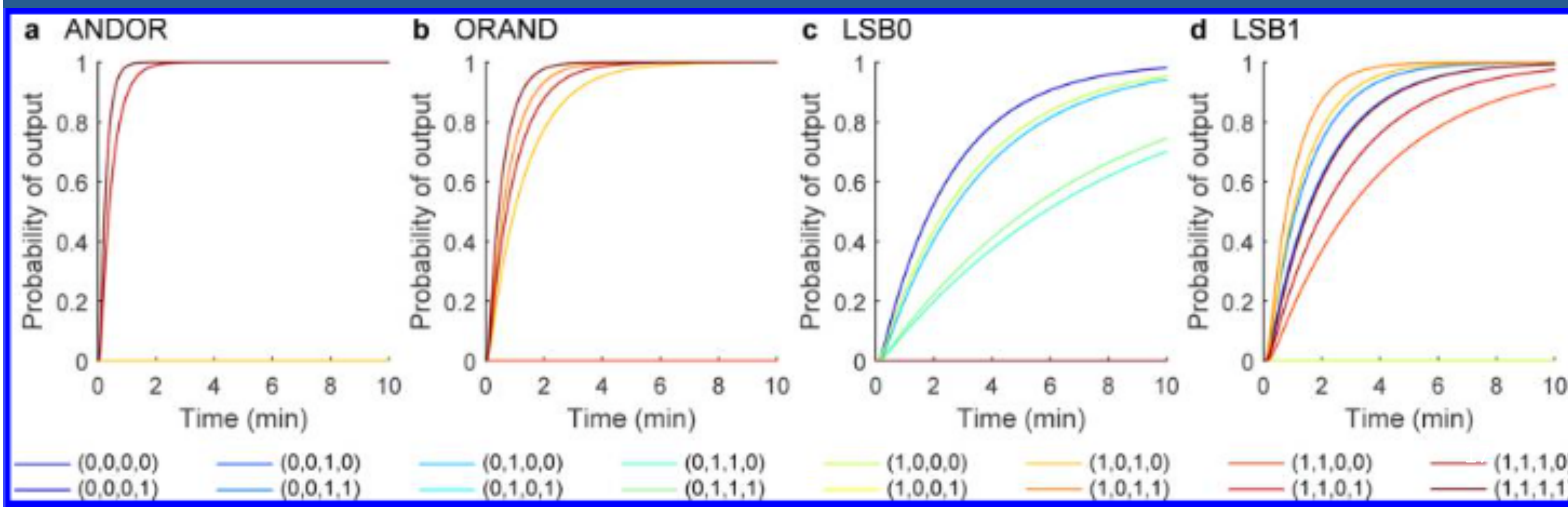


More Complex Circuits

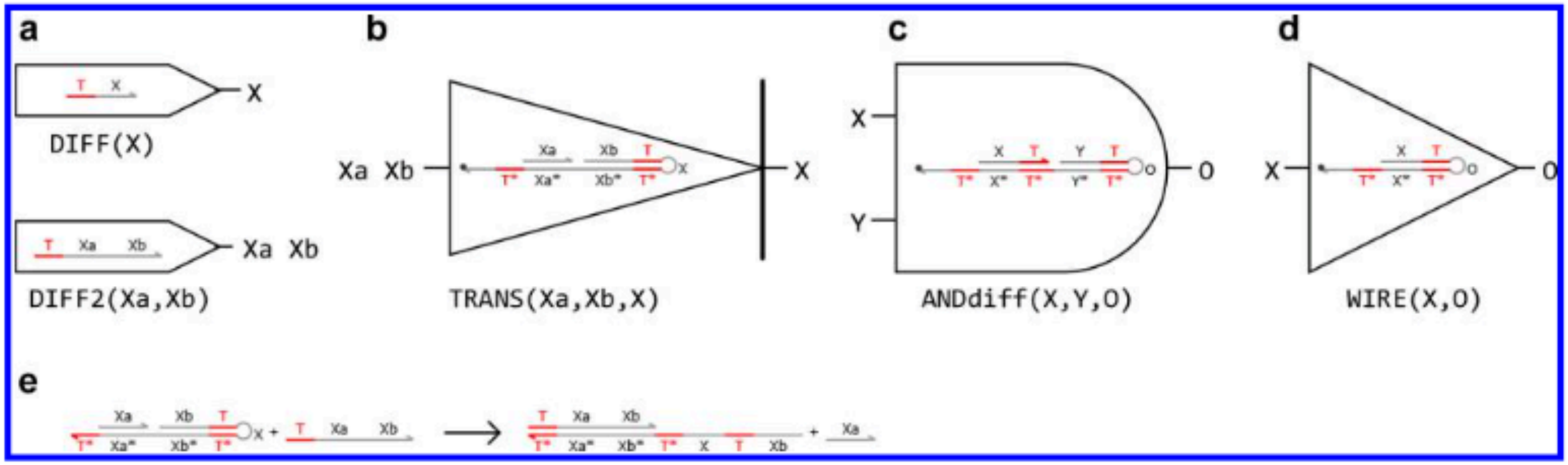




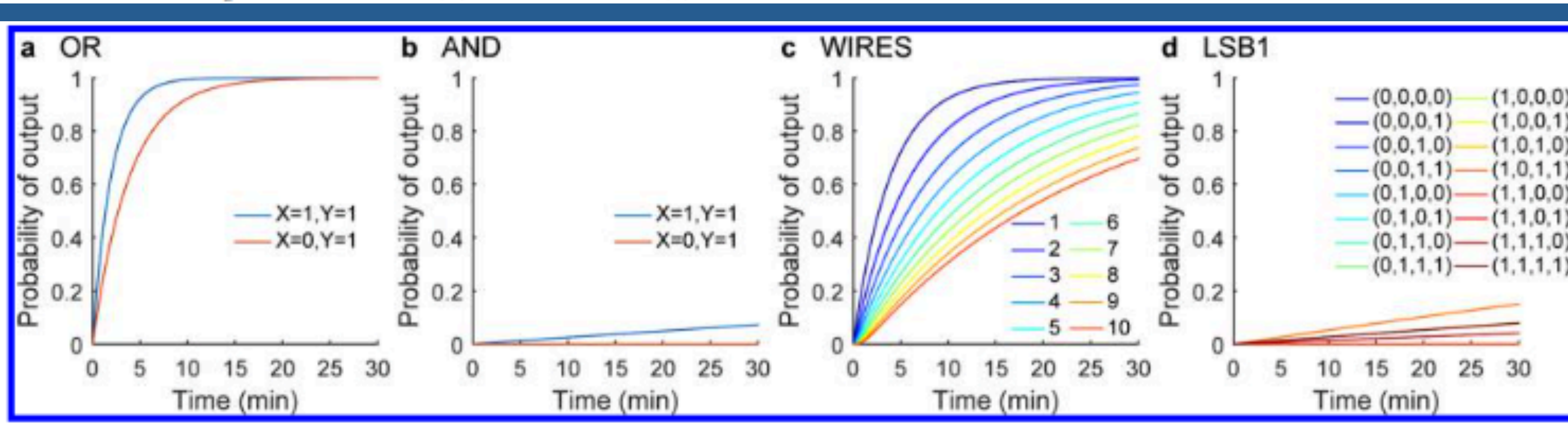
e Layout design (ANDOR)



More Complex Circuits

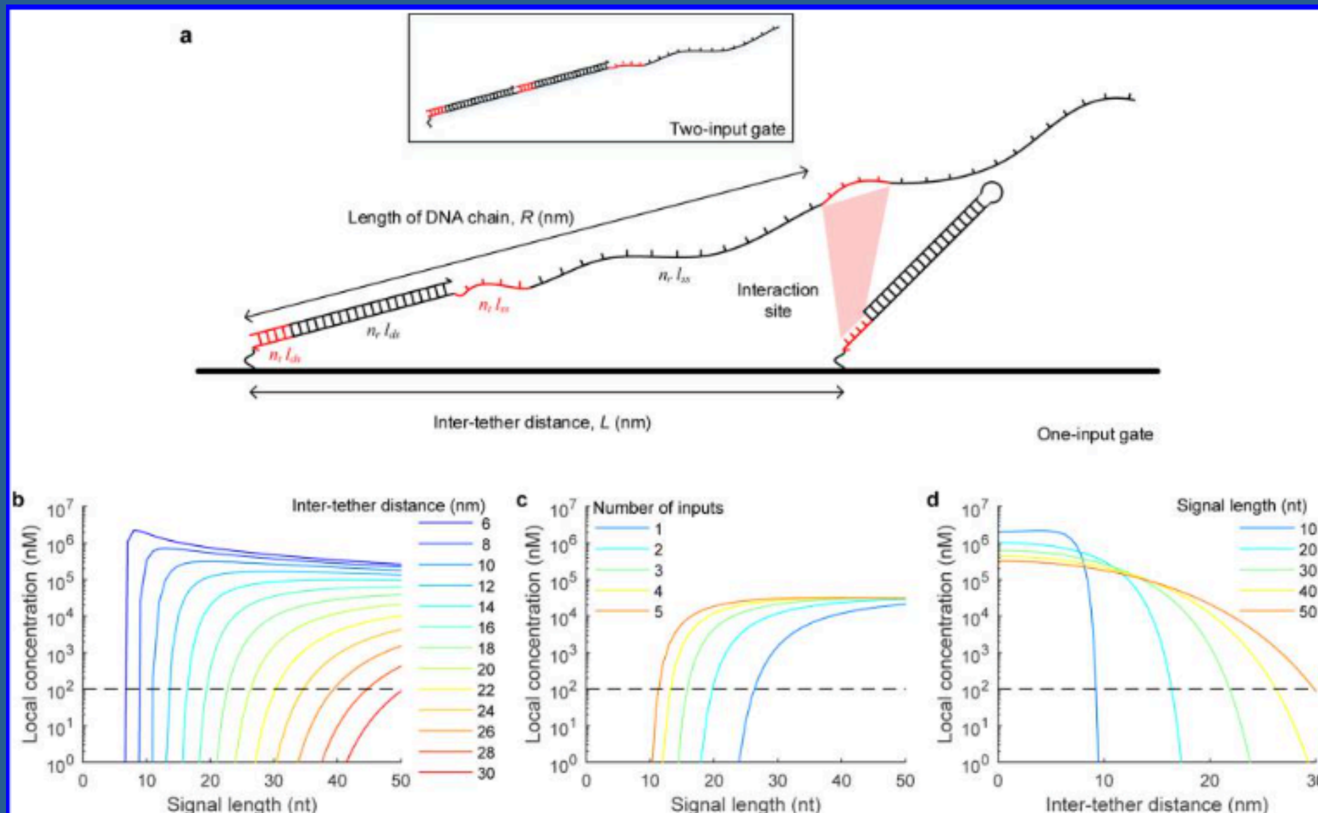


. Diffusible inputs, transducer gate, and diffusible protector strands. (a) Diffusible inputs. (b) Transducer mechanism. Each localized input stand ⟨*TX*⟩ was replaced with a concentration of diffusible strands ⟨TX_aX_b⟩ in solution, together with a single localized transducer complex. (c) AND circuit with diffusible protector strands. (d) WIRES circuit. (e) Behavior of transducer.



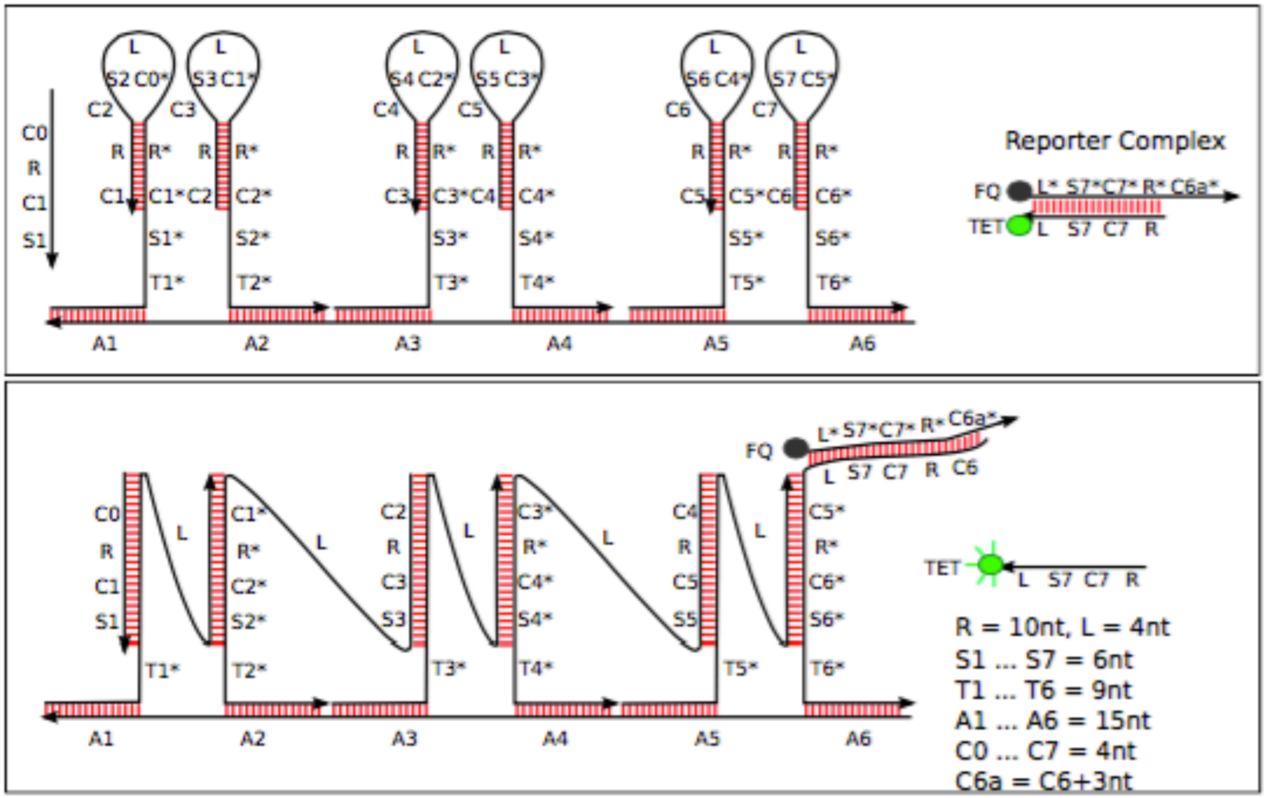
Analysis of localized circuits with diffusible inputs. Probability of circuit output over time, for circuits with diffusible inputs as defined in Figure S1a. All input strands were assumed to be diffusible and at constant concentration c_0 = 100 nM. Models were compiled in Detailed mode with rates as in Figure 1 and $c = 10^4$ nM. The presence of different inputs is indicated in the legends, with 1 corresponding to an input concentration of c_0. A dual rail logic encoding was used for the inputs to LSB1 (Figure 7d). Most of the circuits failed to reach 0.75 probability of completion within an hour.

Simulating Biophysical Model

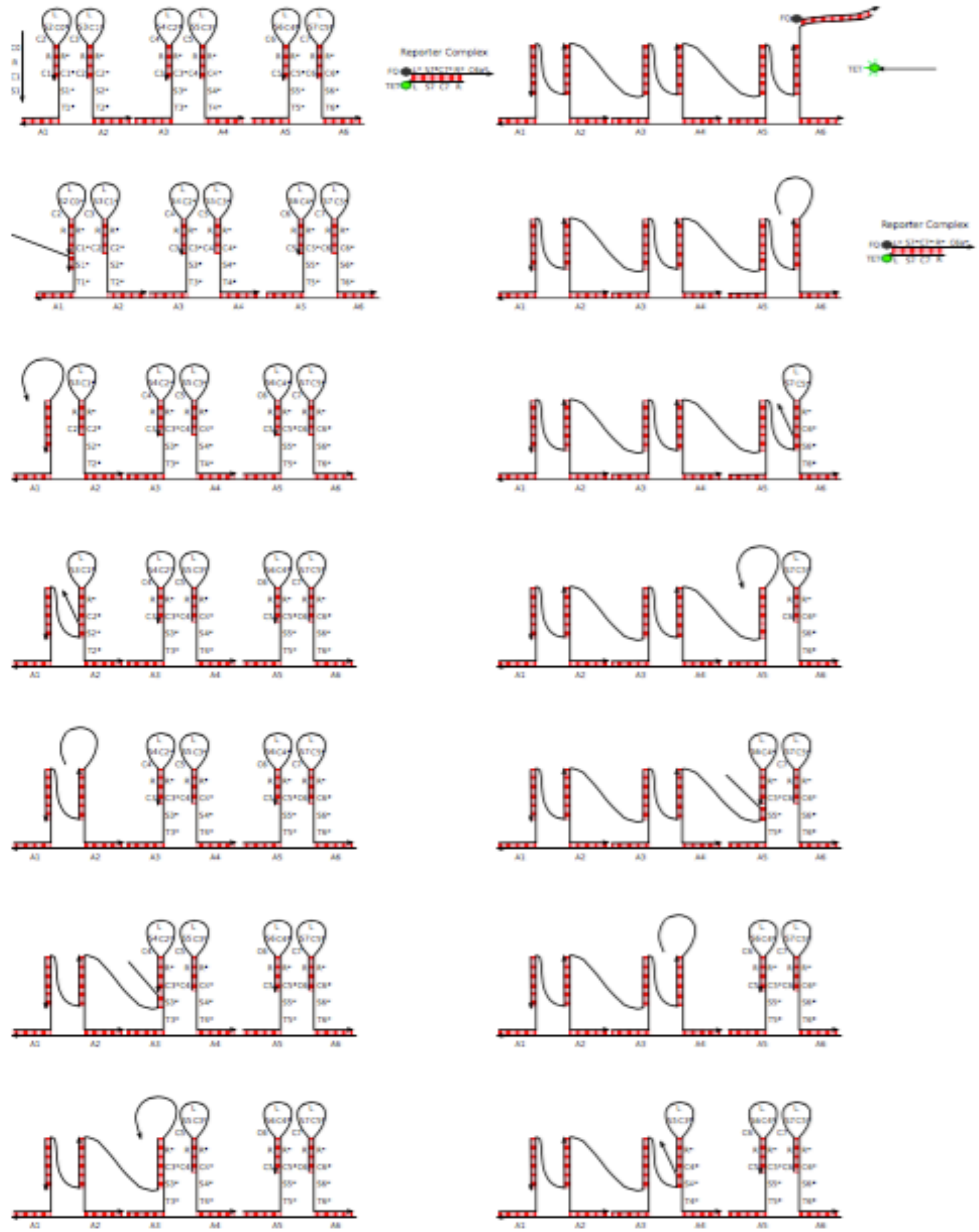


. A biophysical model of tethered hybridization. (a) A graphical depiction of the interaction between an open tethered gate and a nearby (closed) target. Indicated are double- and single-stranded sections on the open tethered gate, which influence its flexibility and thus the local concentration of the interaction. The boxed inset illustrates an open tethered gate that has received two inputs, resulting in a longer double-stranded section. (b–d) Using a worm-like chain (WLC) model, the local concentration is calculated approximately as a function of the number of nucleotides in a signal strand, which defines the length of both the double-stranded portion of the open tethered gate and single-stranded portion upstream of the interaction site. In (b) and (d), a single input strand is assumed and therefore the double-stranded portion is one signal strand long, whereas in (c), the number of input strands to the open tethered gate scales the double-stranded portion. In (c), an intertether distance of 20 nm is assumed.

Localized Hybridization Reactions on Nanotracks

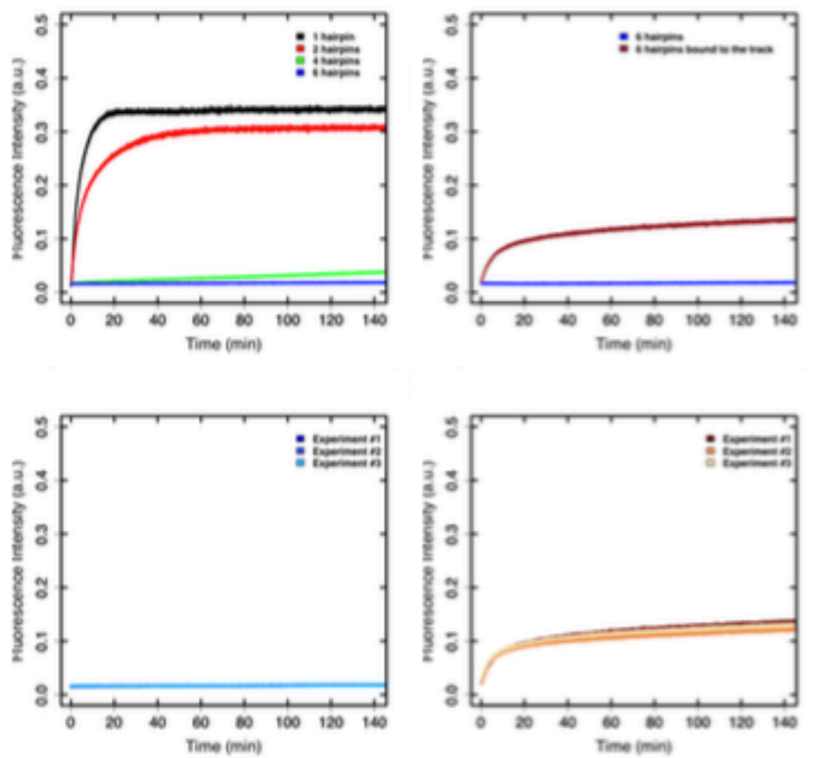


Design of six cascade DNA hairpin gates bound to a long DNA track and the design of cascade strand displacement pathway. The initial (A) and final (B) states of the localized chain reactions are shown, see SI for graphic details of each reaction step. A series of various hairpin gate designs were explored and these hairpin gates for the cascade strand displacement chain reactions were results from three prior hairpin gate designs as shown in SI-S1.



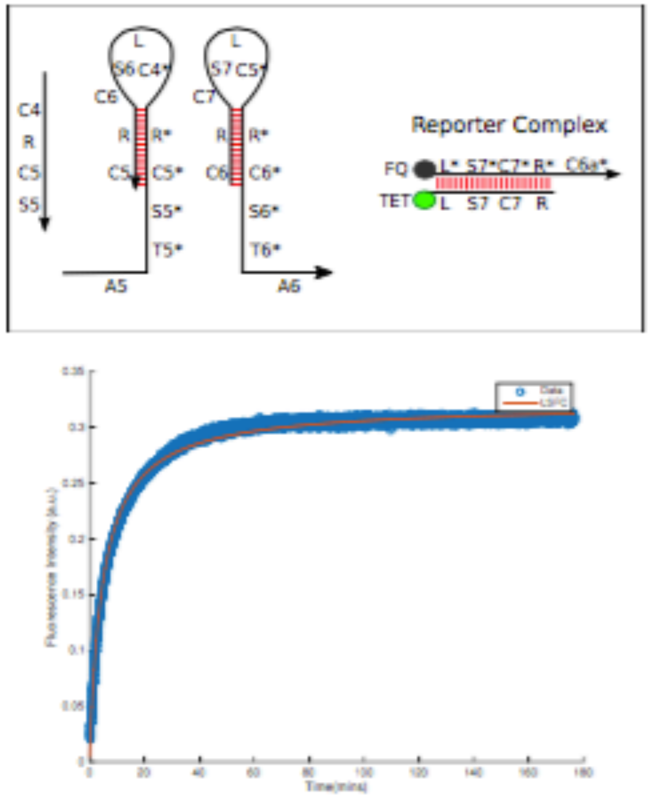
Intermediate states of the cascade strand displacement chain reactions of six hairpin gates bound to the track

Experimental Data: Speedup



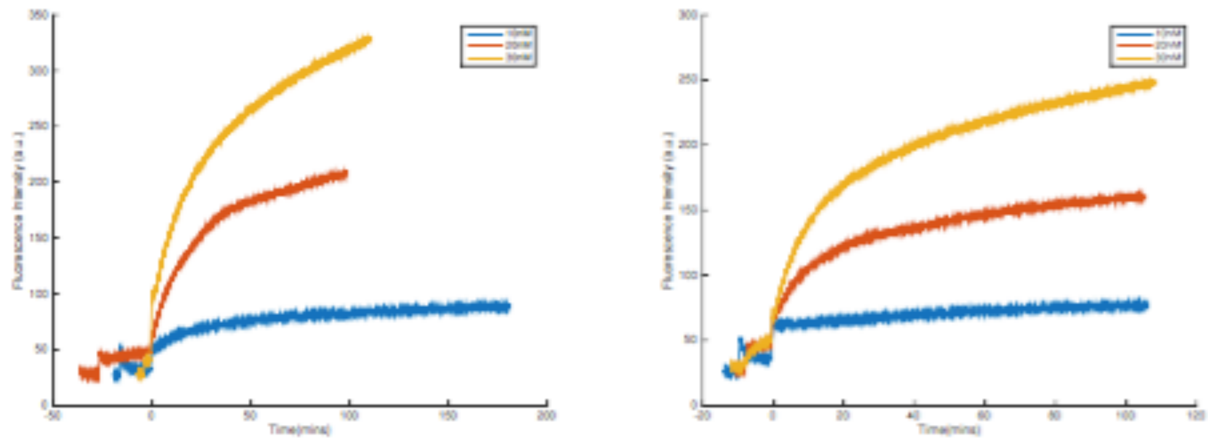
Ensemble fluorescence emission of the locality on the dynamics of DNA hybridization chain reactions - (Top-Left) Cascade strand displacement as a function of hairpin gates without the long DNA track. (Top-Right) Cascade strand displacement as a function of the long track. (Bottom-Left) Reproducibility of cascade strand displacement of 6 hairpin gates. (Bottom-Right) Reproducibility of cascade strand displacement of 6 hairpin gates bound the track. All fluorescence data were normalized with respect to the maximum fluorescence of the reporter complex in the absence of DNA gates.

Experimental Data



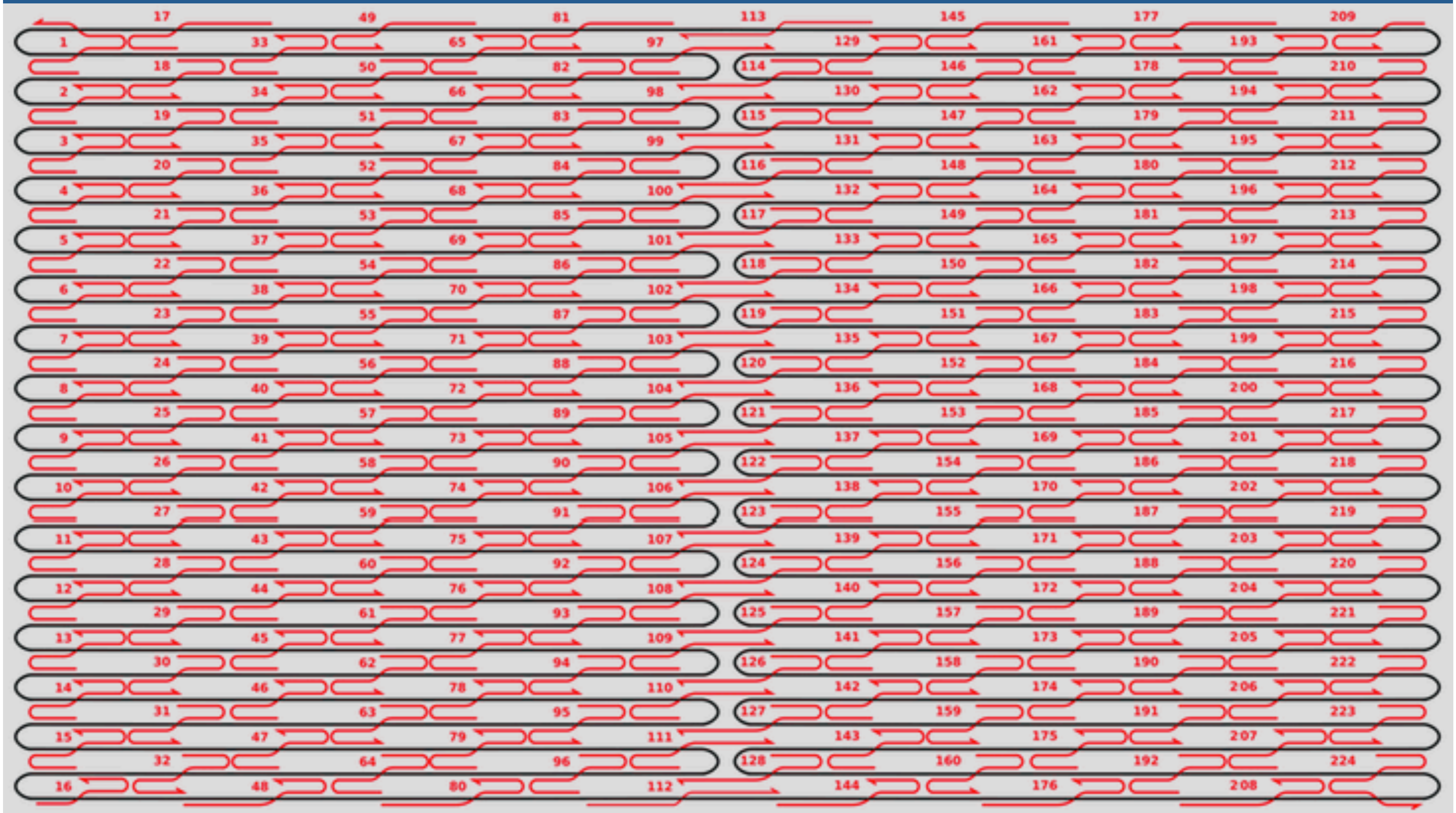
: Fluorescence kinetic data of two hairpin gates in the presence of the initiator (blue) and the fitting data (red) using the Matlab *lsqcurvefit* method

Experimental Data: Concentration Test

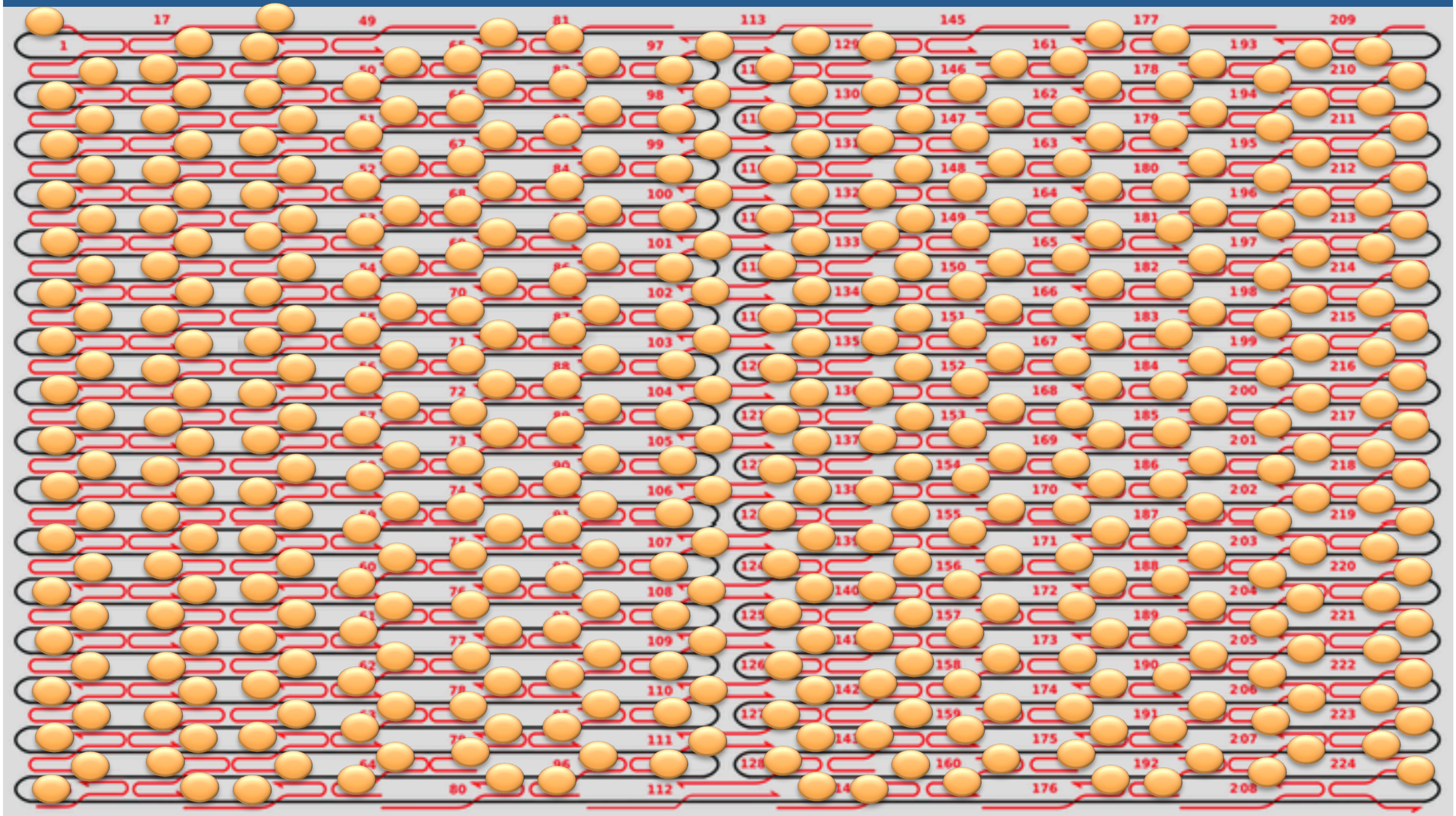


Fluorescence data of six hairpin gates bound to the track as a function of hairpin gates' concentration (Left) and six hairpin gates without the track as a function of hairpin gates' concentration (Right).

Localized Hybridization Reactions on DNA Origami



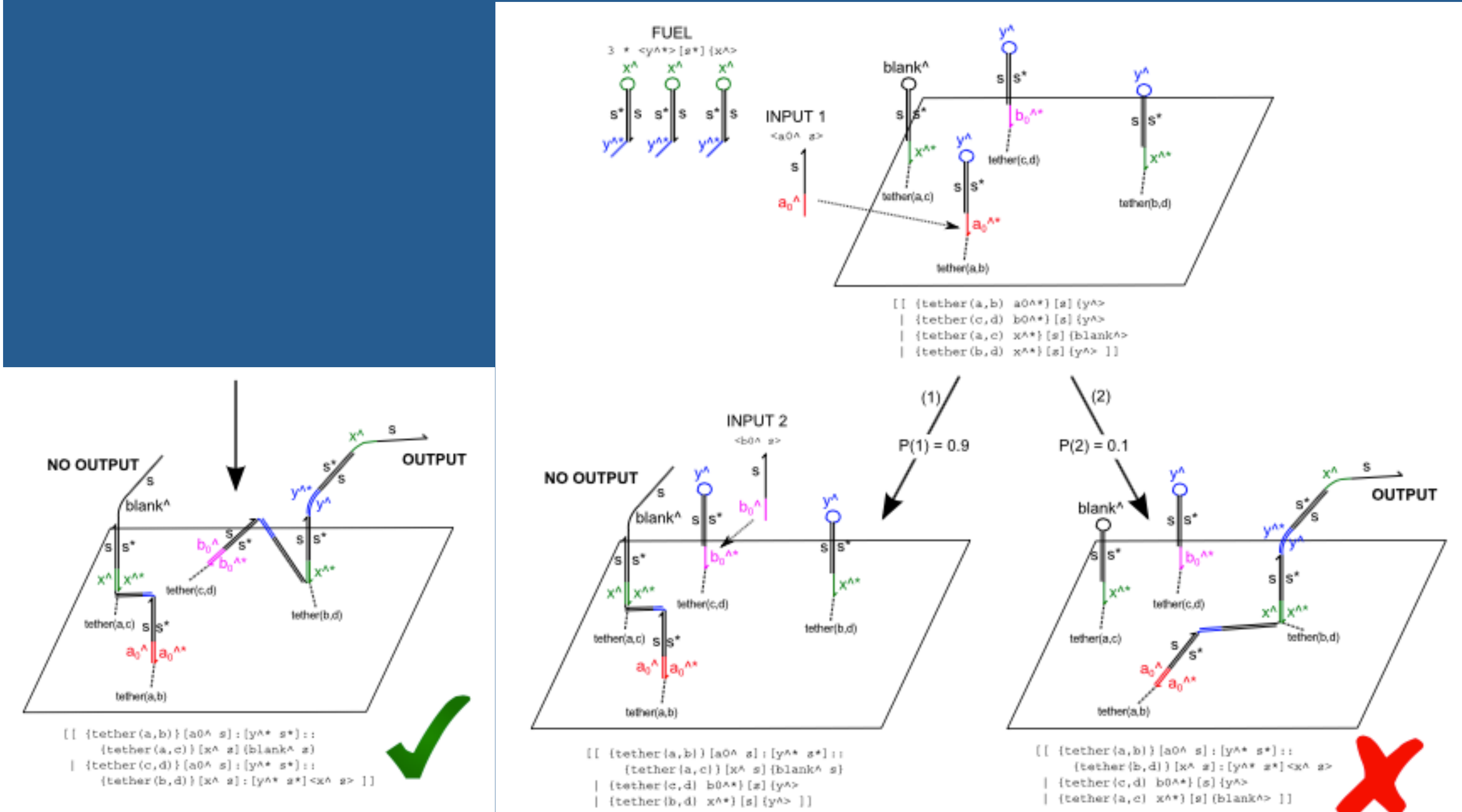
224 x 2 binding sites



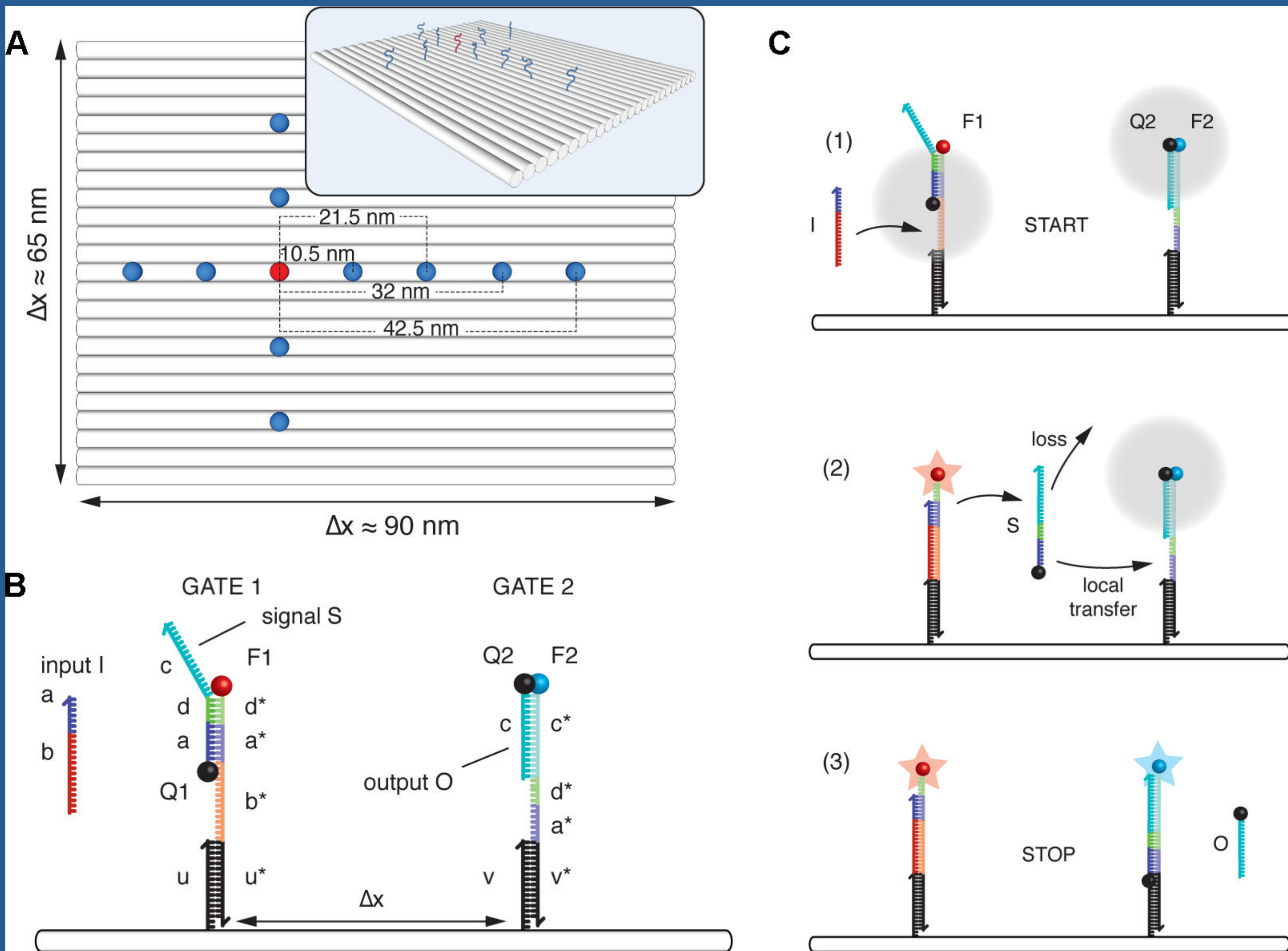
Practical Considerations

- Errors of Assembly:
  - Staples are missing 7% of the time.
  - => Only 70% of 4 gates will be formed.
- Dealing with Errors of Assembly
  - Built-in redundancy.
  - Consensus methods.
- Hairpin Issues:
  - Hairpins interacting during anneal processes.
  - Hairpin structure is stable at a higher temperature.

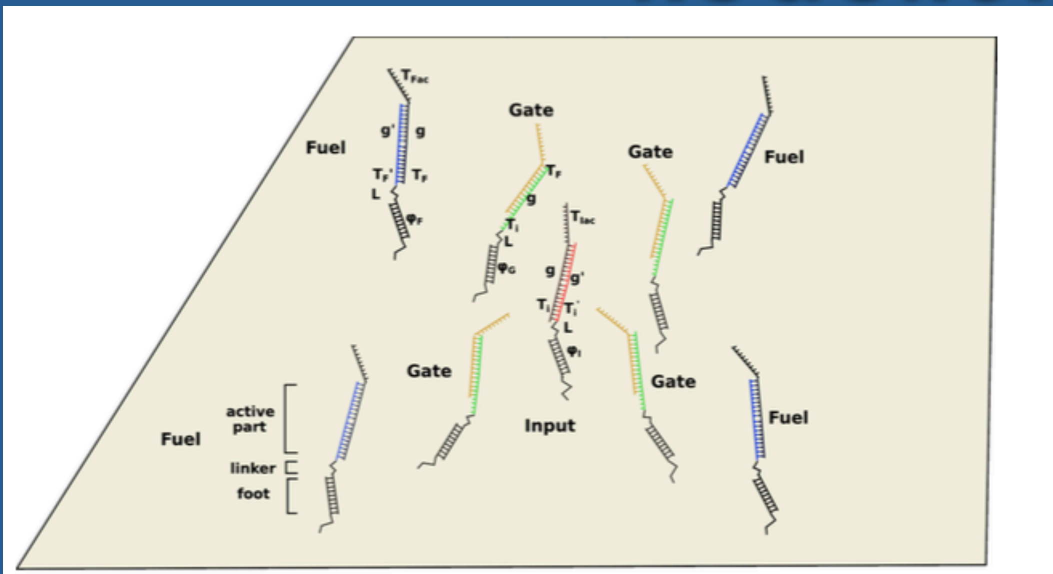
Abstract Modeling of Tethered DNA Circuits

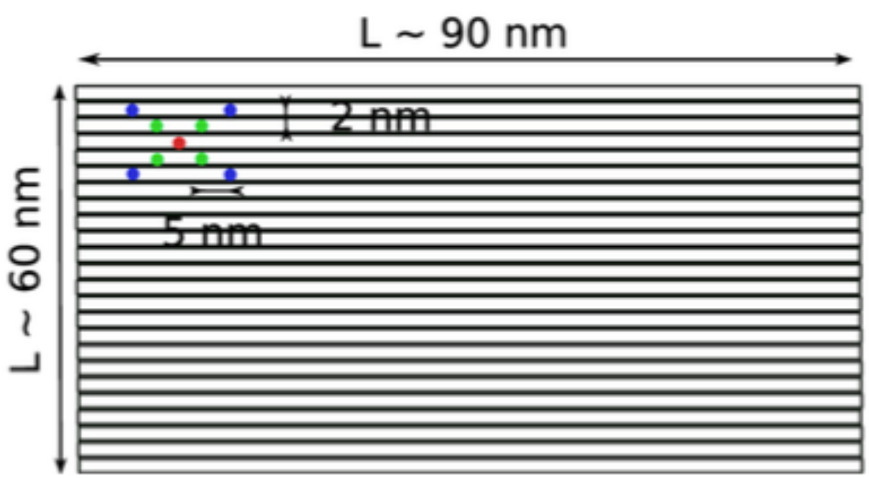


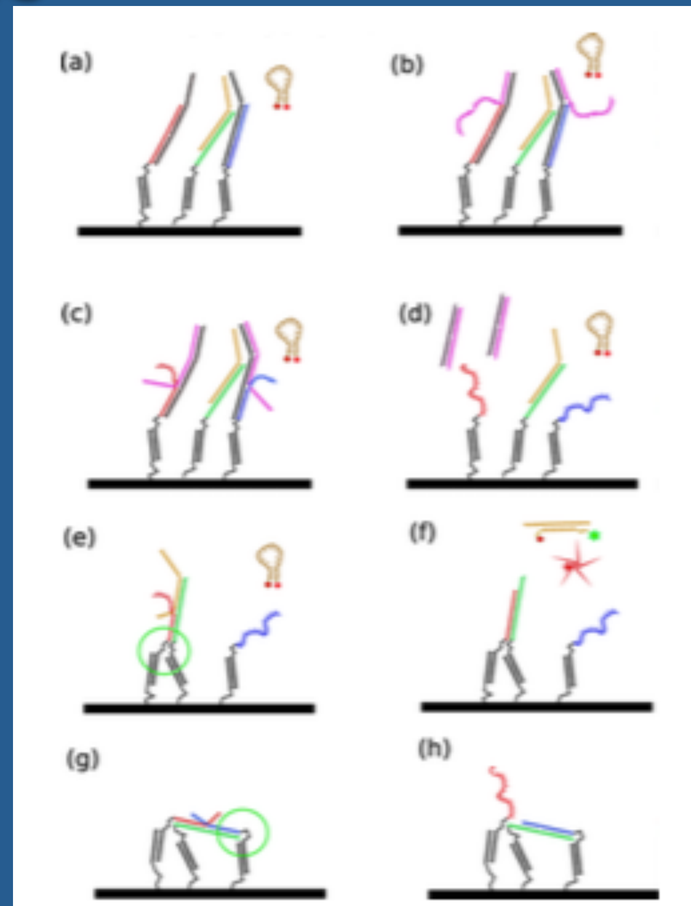
Robustness of Localized DNA Strand Displacement Cascades



Connecting Multiple Distinct Localized DNA Strand Displacement Reactions







Diffusive transport of molecular cargo tethered to a DNA origami platform